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Aggression in replacement grower and finisher gilts fed a short-term high-tryptophan diet and the effect of long-term human–animal interaction

Rosangela Poletto [a,b], Robert L. Meisel [c,1], Brian T. Richert [b], Heng-Wei Cheng [a], Jeremy N. Marchant-Forde [a,*]

[a] USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN 47907, USA
[b] Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
[c] Department of Psychological Sciences, Purdue University, West Lafayette, IN 47906, USA

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ABSTRACT

Aggression can be a major problem for swine production as it negatively impacts the pigs' health and welfare. Increasing tryptophan (TRP) intake to raise brain serotonin (5-HT)—key for aggression control, and long-term positive social handling can reduce stress in pigs. Objective was to feed a short-term high-TRP diet to grower (3 months) and finisher (6 months) maternal gilts that were either socially handled or not and measure their behavioural activity and aggressiveness. Eight pens of six unrelated gilts were split into two blocks balanced for litter, social handling (non- vs. handled) and dietary treatment (control vs. high-TRP). Social-handling was applied three times per week, from day 45 until 6 months of age. At 3 months, two handled and two non-handled pens were assigned to control while the other four pens were assigned to the high-TRP diet fed *ad libitum* for 7 days (days 1–7). At 6 months of age, pen assignment to dietary treatments was swapped. Body weights and blood were taken at days 1 (pre-feeding) and 7. Blood samples were analyzed for TRP and 5-HT concentrations using high pressure liquid chromatography. Behaviour was recorded from days 1 to 5 and scan-sampling used to determine time-budget behaviours and postures in a 12-h period each day (06:00–18:00 h). Aggression evaluation in the home pen focused on counts of agonistic interactions, bites and head-knocks per interaction during three, 30-min intervals (08:00, 12:00, and 16:00 h) from days 1 to 5. Resident–intruder (R–I) test was carried out for a maximum of 300 s at days 6 and 7 to measure aggressiveness, predicted by the latency to the first attack and attack outcomes. A 2×2 factorial arrangement of dietary treatment and social handling within age was analyzed by repeated measures of mixed models and Tukey adjustments. The TRP-added diet raised blood TRP concentration of 3- and 6-month-old gilts by 180.7% and 85.2% respectively ($P < 0.05$), reduced behavioural activity and time spent standing, while increasing lying behaviour, mostly in grower gilts ($P < 0.05$). High-TRP diet reduced the number of agonistic interactions, and aggressiveness in 3-month-old gilts, which took longer to attack the intruder pig, and displayed fewer attacks on the first day of testing ($P < 0.05$). Long-term positive social handling improved growth performance and had a slight effect on behaviour ($P < 0.05$). Provision of enhanced TRP diet reduced behavioural activity and aggressiveness of grower gilts, and these results are likely mediated by activation of brain serotonergic system. Short-term high-TRP dietary supplementation may be used to reduce aggression at mixing in young pigs.

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* Corresponding author at: USDA-ARS, LBRU, Purdue University, 125 S. Russell St., West Lafayette, IN 47907, USA. Tel.: +1 765 494 6358; fax: +1 765 496 1993.
E-mail address: Jeremy.Marchant-Forde@ars.usda.gov (J.N. Marchant-Forde).
[1] Present address: Department of Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA.

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1. Introduction

Aggression is one of the most significant problems when pigs are housed in groups. In a group context, pigs naturally engage in aggressive interactions when introduced to unfamiliar conspecifics to determine their social hierarchy status (Mendl et al., 1992; D'Eath and Turner, 2009) and, thereafter, use aggression to gain access to resources such as feed (Marchant-Forde, 2002). Ordinarily in a stable group, levels of aggression should be relatively low. However, deficiencies in system design or repeated changes in group composition can result in persistent aggression, which may lead to physical injury, including lameness and acute stress. If unresolved, persistent aggression may cause chronic stress with negative consequences such as increased disease susceptibility and impaired productivity (Straw et al., 2006).

The cerebral neurotransmitter serotonin (5-HT) is essential for aggression inhibition (Nelson and Chiavegatto, 2001) and also regulates sleeping, appetite, mood, and behavioural activity (Sève, 1999; Linder et al., 2007). Tryptophan (TRP), an essential amino acid only acquired through diet, is the precursor for 5-HT and because TRP can cross the blood–brain-barrier, dietary elevations of TRP have been applied in an attempt to reduce stress in group housed pigs (Adeola and Ball, 1992). Ingestion of elevated amounts of TRP leads to increased TRP concentration in the blood, which positively and directly correlates with brain 5-HT availability in pigs (Fernstrom, 1974; Meunier-Salaün et al., 1991; Henry et al., 1992; Adeola and Ball, 1992).

Human–animal interactions in a production setting are an important component of daily management that can have major consequences on the animals' behavioural response to stress. Pleasant human handling minimises the stress from routine practices and improve the animals' health and productivity (Hemsworth and Coleman, 1998; Hemsworth, 2007). Positive gentle handling of gilts increases growth rate (Hemsworth et al., 1981) and may play a role in reducing inter-individual aggression (Popova et al., 1993). Popova et al. (1991) have demonstrated that human handling of silver foxes is directly related to activation of the serotonergic system in the brain (higher concentrations of 5-HT and its metabolite, and greater TRP hydroxylase activity).

Thus, we hypothesised that feeding a high-TRP concentration diet for 7 days would decrease motor activity and reduce aggression in grower and finisher replacement gilts, and that an effect of long-term pleasant social handling would also be seen on behavioural activity and potentially on aggression. The resident–intruder (R–I) test employed in this study is a standard behavioural method that measures individual pig's aggressiveness in a controlled and standardised context and is intended to predict aggression when mixed in a group (Erhard and Mendl, 1997; D'Eath, 2002). Both, positive social handling and high-TRP feeding enhance feed intake (Hemsworth et al., 1981) and, consequently, growth performance of pigs (Henry et al., 1992). Therefore, the objectives of this study were to determine the effects of short-term feeding of a high-TRP concentration diet and pleasant social handling on average daily gain (ADG), blood concentration of TRP and 5-HT, time-budget behavioural activity, and aggression in the home pen and during a R–I test in grower (3-month old) and finisher (6-month old) maternal gilts.

2. Material and methods

2.1. Animals and housing

Experimental gilts were selected according to litter and were assigned to one of two blocks of 24 maternal line gilts ([US York × Landrace] × [US York]) in each block. The experiment was set as a complete balanced block design with two identical blocks determined by date of birth. Six litters per block were used and each litter was represented by four gilts ($n = 48$). Block 1 was born and went on trial 2 months prior to block 2. Experimental gilts were randomly selected from each litter, although physically impaired piglets (e.g. severe lesions or low body weight) were avoided. Maternal line gilts were chosen because of a parallel pilot study with the same experimental gilts investigating the effects of long-term pleasant social handling on sow productivity parameters.

Following birth, gilts were housed with siblings in standard farrowing enclosures (1.5 m × 2.7 m) with sow crates (0.6 m × 2.2 m) in the center of the pen. All piglets were provided with one heat lamp (250 W) and one heat mat (0.3 m × 1.2 m) placed in the farrowing enclosures, and received standard processing procedures (body weighing, ear notches, tail docking, teeth clipping, and iron supplement by intramuscular injection) from the Purdue Swine Unit staff at an approximately 3 days of age. Fostering among experimental litters was not allowed. If litter size was greater than 10 piglets at birth, then the additional piglets in that litter were removed and fostered on to non-experimental litters. Weaning was carried out at an average of 19.1 ± 1.4 days of age (mean ± SEM). At weaning, experimental gilts were allocated into eight pens (four pens per block) in a manner that each set of four littermates were separated among pens within each block. Thus, each pen housed six non-related gilts. Nursery pens measured 1.7 m long × 2.0 m wide, and had a nipple drinker and one feeder (0.8 m wide) with five feeding spaces with nursery diet available *ad libitum*. Piglets were kept on 24-h continuous light for the first week then moved to a 12-h light/12-h dark cycle. The initial nursery room temperature was 28.9 °C and dropped 1.3 °C per week until approximately 22.2 °C when piglets were moved to the grow-finish barn.

All gilts remained in the nursery room until 54 days of age and then were moved to the grow-finish barn. The same group of six gilts in each pen was maintained when moving from the nursery into the eight new pens in the grow-finish barn, and thus the gilts were not mixed with any unfamiliar pigs at that time. The new grow-finish home pens measured 4.3 m long × 1.7 m wide, and contained a nipple drinker and one single-space feeder (0.5 m wide). The temperature in the barn was maintained at 18.5 °C. Gilts were kept on a 12-h light/12-h dark cycle and received corn-soybean meal (48% crude protein) basal diets *ad libitum* according to the appropriate growth stage (grower I and II, finisher I and II) unless otherwise stated.

Gilts remained under these housing conditions until the end of the experiment, when they were 6 months of age. This study was approved by the Purdue University Animal Care and Use Committee and animals were housed in accordance with FASS (1999) guidelines. The current project was funded by the United States Department of Agriculture-Agricultural Research Service under the National Program 101.

2.2. Human social handling

Gilts housed in four of the eight experimental pens belonging to blocks 1 and 2 were subjected to pleasant social handling (handled pens) while the other four pens were left alone (non-handled pens; Table 1). Handled pens, when in the nursery room, were adjacent to each other permitting the handler to move towards the rear wall away from the non-handled pens. In the grow-finish barn, the handled (two per block) and non-handled (two per block) pens were alternated and also separated by one pen of non-experimental pigs. The social handling was carried out at 12:00 h ($\pm$1 h) on three alternate days every week, and started when gilts were 45 days of age and lasted until 6 months of age. The human social handling consisted of a person, the handler, who slowly and quietly entered and walked in the assigned pen, allowing the gilts to voluntarily approach her. Gilts in close range of the handler were gently patted while the handler used a quiet voice to signal her presence in the pen. A timer was used to record the duration of social handling; a total of 6 min was spent in each handled pen from 45 days until gilts were 3 1/2 months of age (14 weeks of age). From this latter time until when gilts were 6 months of age (24 week of age), the time the handler spent socially handling the gilts was shortened to a total of 3 min spent per pen.

2.3. High-tryptophan diet feeding

A split-plot design was adopted for the feeding trial. At both 3 and 6 months of age, two handled and two non-handled pens from each block were fed a high-TRP diet while the other two handled and two non-handled pens were fed a CTL diet. Gilts that received the control (CTL) diet at 3 months of age in turn received the high-TRP diet at 6 months of age, and vice versa (Table 1). To produce the high-TRP diet, supplemented TRP concentration was raised while the concentrations of other large neutral amino acids (LNAA) were maintained, hence increasing the TRP:LNAA ratio in the experimental diet provided. Furthermore, concentration for LNAA was equal for the CTL and high-TRP diets. Diets provided to gilts were in accordance with the appropriate growth stage (grower and finisher) and followed the National Research Council (1998) requirements. Samples from the diets provided to the experimental gilts at both ages were collected and ground through a 1 mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, USA) prior to analysis. Amino acid content was determined at the University of Missouri via AOAC Method # 982.30E (AOAC, 2000). Details on the calculated and analyzed diet compositions fed to the experimental gilts are presented in Table 2.

For each age group (3 and 6 months), the feeding trial lasted a total of 7 days (days 1–7). At day 1 (approximately at 10:00 h), any remaining feed in the feeders was removed and the high-TRP (high TRP:LNAA ratio) or CTL (normal TRP:LNAA ratio) diets were delivered to the assigned pens and made available *ad libitum*. Gilts assigned to the CTL dietary treatment received a corn-soybean meal basal diet, while the high-TRP diet was formulated so that TRP was fed at a 250% inclusion rate of the TRP concentration found in the CTL diet (Table 2). The percentage inclusion rate for TRP was determined based on a pilot study carried out with gilts at the ages of interest for the current study, and previous studies that used a similar dietary experimental design (e.g. Adeola and Ball, 1992; Koopmans et al., 2005; Li et al., 2006). Social handling was not performed during the week of the feeding trial. All gilts were weighed immediately prior to starting the feeding trial (day 1) and on the last day of the feeding trial (day 7) to determine pre- and post-feeding trial body weights and calculate the average daily gain (ADG) of body weight for each pen.

2.4. Time-budget behaviour

All gilts in each pen were individually marked with stock-marker and behaviour was continuously recorded for 12 h (06:00–18:00 h) from the start of the dietary treatment and continued for 5 consecutive days (days 1–5), yielding a total of 5×12 h behaviour recordings. The gilts' behavioural activities were recorded in real time using ceiling-mounted cameras (Panasonic WV-CD110AE, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) that

Table 1
Experimental design summary for the pleasant social handling protocol and short-term high-tryptophan feeding trial.

	Age of maternal gilts	Experimental block[a]			
		Pen 1	Pen 2	Pen 3	Pen 4
Pleasant social handling[b]	45 days to 6 months	Handled	Non-handled	Handled	Non-handled
Short-term	3 months	High-TRP	Control	High-TRP	Control
Feeding trial[c]	6 months	Control	High-TRP	Control	High-TRP

[a] The split-plot experimental design described for block was applied to both blocks 1 and 2, which are identical replicates from each other. Gilts in block 1 were born and went on trial 2 months prior to block 2. There were six litters per block and each litter was represented by four gilts; each set of four littermates was equally divided among the four pens (1 gilt per pen) within each block.

[b] Social handling was performed 3 days per week, starting when gilts were 45 days and lasted until they were 6 months old. From 45 days to 3 1/2 months, the handling of gilts lasted 6 min per pen per day; from 3 1/2 until 6 months of age, handing of gilts lasted 3 min per pen per day.

[c] Both diets were fed to the experimental gilts *ad libitum* for 7 days. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet.

Table 2
Composition of the control and high-tryptophan diets provided to the grower (3 months of age) and finisher gilts (6 months of age) during a 7 day feeding trial.

Ingredient, %	Grower phase diet		Finisher phase diet	
	Control	High-TRP	Control	High-TRP
Ground corn	81.90	81.90	91.66	91.66
Soybean meal, 48% CP	13.84	13.84	4.78	4.78
Choice white grease	1.00	1.00	1.00	1.00
Ground limestone	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.66	0.66	0.34	0.34
Vitamin premix[a,b]	0.15	0.15	0.10	0.10
Trace mineral premix[c,d]	0.14	0.14	0.10	0.10
Phytase[e] (600 FTU/g)	0.08	0.08	0.08	0.08
Salt	0.35	0.35	0.25	0.25
Antibiotic[f]	0.10	0.10	0.03	0.03
L-Lysine–HCL	0.30	0.30	0.30	0.30
DL-Methionine	0.04	0.04	0.01	0.01
L-Threonine	0.11	0.11	0.10	0.10
L-Tryptophan[g]	0.03	0.34	0.03	0.26
Starch[g]	0.31	0.00	0.23	0.00
Calculated analysis				
ME, kcal/kg	3360.70	3360.70	3384.70	3384.70
CP, %	13.31	13.58	9.75	9.94
Lysine, %	0.85	0.85	0.60	0.60
Tryptophan, %	0.16	0.46	0.11	0.33
Ileal dig. tryptophan, %	0.12	0.42	0.09	0.30
Ca, %	0.60	0.60	0.50	0.50
Available P, %	0.24	0.24	0.16	0.16
Analyzed composition				
CP, %	13.49	13.98	10.79	11.08
Lysine, %	0.86	0.85	0.64	0.69
Tryptophan[h], %	0.14	0.35	0.13	0.31

[a] Provided per kg of grower diet: vitamin A, 3638 IU; vitamin D3, 364 IU; vitamin E, 26.4 IU; menadione, 1.20 mg; vitamin B12, 21 μg; riboflavin, 4.26 mg; pantothenic acid, 13.20 mg; and niacin, 19.80 mg.
[b] Provided per kg of finisher diet: vitamin A, 2426 IU; vitamin D3, 242 IU; vitamin E, 17.6 IU; menadione, 0.80 mg; vitamin B12, 14 μg; riboflavin, 2.84 mg; pantothenic acid, 8.80 mg; and niacin, 13.2 mg.
[c] Provided per kg of grower diet: Fe, 87.3 mg; Zn, 87.3 mg; Mn, 10.8 mg; Cu, 8.1 mg; I, 0.32 mg; Se, 0.30 mg.
[d] Provided per kg of finisher diet: Fe, 48.5 mg; Zn, 48.5 mg; Mn, 6.0 mg; Cu, 4.5 mg; I, 0.18 mg; Se, 0.30 mg.
[e] Provided per kg of diet: 435 FTU (Natuphos, BASF, Florham Park, NJ).
[f] Grower phase antibiotic provided per kg of diet—110 ppm chlortetracycline (Aureomycin 50, Alpharma Inc., Fort Lee, NJ). Finisher phase antibiotic provided per kg of diet 22 ppm tylosin (Tylan 40, Elanco, Indianapolis, IN).
[g] L-Tryptophan (Ajinomoto Heartland LLC, Chicago, IL) added at the expense of starch to create diets targeting 250% of tryptophan (TRP) concentration available in the control diet.
[h] The analyzed TRP for the grower phase grower diet composition reaches 250.0% of the TRP in the control diet, while the analyzed TRP for the finisher phases reaches 238.5%.

were attached to a digital video recording system (IPD-DVR816, Inter-Pacific, Inc., Northbrook, IL). No software was used to extract the behaviours; an observer, the same person, watched all recorded videos. A 10-min, scan-sampling method was used to evaluate time-budget of behaviours and postures of gilts in each pen. Inactive and active behaviours (all behaviours excluding time spent inactive), and postures in which the gilts were observed, along with their descriptions, are presented in the ethogram described in Table 3. The only posture and behaviour combinations that were not possible were lying + walking, sitting + walking and lying + drinking. Data from all gilts in the pen were then combined to generate a behavioural time-budget profile for the group of pigs in each pen. Proportions of time spent performing each behaviour and in a specific posture were averaged by day of observation. Means of time-budget behaviours and postures are shown in the text and tables as percentages (%) of time spent performing the behaviours and in specific postures for a 12-h period.

2.5. Home pen aggression

To evaluate the occurrence of agonistic interactions taking place in the home pen, and the number of bites and head-knocks displayed during each interaction, the video data were also analyzed using all-instances sampling. Behavioural observation focused on social interactions (i.e., agonistic encounters) and on the individual interaction components displayed by the two pigs engaged in the agonistic interaction. These observations were carried out for a total of 5 days (days 1–5), during three time periods of 30 min each day (08:00–08:30 h; 12:00–12:30 h; 16:00–16:30 h). These time intervals were chosen in order to sample representative behavioural activity during the 12-h recorded period (06:00–18:00 h) that encompassed morning, midday, and afternoon. The total number of agonistic interactions and the number of offensive components such as bites and head-knocks displayed during each interaction were recorded. An agonistic interaction was defined as an event in which one or both pigs directed one or a sequence

Table 3
Ethogram used for evaluation of the time-budget behaviours and postures observed in the growing gilts.

Item	Description
Behaviour	
Inactive	Physical immobility without any activity
Alert	Ears erect in a position of attention
Walking	Walking or running around the pen
Nosing/rooting	Manipulating with snout any item of the pen (e.g. floor, walls)
Sham-chewing	Chewing action performed without the presence of food in the mouth
Non-aggressive interaction	Directing investigatory behaviour towards another pig (e.g. sniffs, massage)
Aggressive interaction	Engaging in agonistic interaction—pushing, biting and head-knocking with another pig
Drinking	Drinking water from the nipple drinker
Feeding	Head positioned inside of the feeder with oral movement
Eliminating	Urinating or defecating
Posture	
Standing	Standing on all four legs
Lying	Lying down, either on belly or on one side
Sitting	Dog-sitting with rump on the floor and shoulders raised up with front legs extended

of aggressive behaviours. The initiator of the first attack was recorded. The sum of each component, as well as their combined sum (total number of bites + head-knocks) were divided by the total number of agonistic interactions occurring in each of the 30-min intervals, and summed within each day of observation to determine the average number of each action per agonistic interaction. These averages and the total number of agonistic interactions displayed during each day of the 5 days were then used for statistical analysis.

2.6. Blood collection and HPLC assay

Blood samples were collected from all gilts in each pen at both 3 and 6 months of age. Gilts were sampled immediately before the start of the dietary treatment (day 1 at 09:00 h), and on the last day of the dietary trial (day 7 at 13:00 h). Measures from sampling at day 1 were used as baseline. Samples were collected into 10 mL ethylenediaminetetraacetic acid-coated (K_3EDTA) tubes from jugular venipuncture and 0.3 mL aliquots of blood were stored at $-80\,^{\circ}$C until processing. To measure the peripheral concentrations of TRP and 5-HT, aliquots of the whole blood from individual gilts were acidified using 100 μL 4 M perchloric acid and freshly prepared ascorbic acid. After centrifugation at $13{,}000 \times g$ for 5 min at $4\,^{\circ}$C, the acid supernatants were filtered and transferred to high pressure liquid chromatography tubes then placed into an autosampler set at $4\,^{\circ}$C. Each sample was injected twice onto a Linear FLUOR LC-305 and Column Platinum EPS C-18 with pore size 100 A and 250 mm in length; the mobile phase flow rate was set at 1.2 mL/min. The concentrations (μg/mL) of TRP and 5-HT were calculated from a reference curve made using commercially available standards for TRP and 5-HT (Sigma–Aldrich, St. Louis, MO, USA). Respectively, the inter- and intra-assay coefficients of variation for TRP were 4.1% and 3.0%, and for 5-HT were 4.1% and 2.4%.

2.7. Resident–intruder (R–I) test

All gilts in each experimental pen, at both 3 and 6 months of age, were resident pigs in the R–I tests, which were carried out to measure the gilts' aggressiveness. At both ages, gilts were subjected to R–I test training sessions that took place during the week prior to testing, allowing them to become acquainted with the testing arena that was set within their home pens. Pen mates were moved as pairs into the arena for 300 s on three alternated days during the training week, with pairs being interchanged each day. The test arena was formed using a solid white, opaque acrylic mobile panel that divided the home pen in half, walling the back and both lateral sides of the pen (Fig. 1). The front of the pen (i.e., gate) was maintained uncovered during training and testing periods. The panel prevented visual, physical or social contact of the gilts being tested with pen mates and pigs from neighboring pens, thus preventing any possible interference with the R–I test.

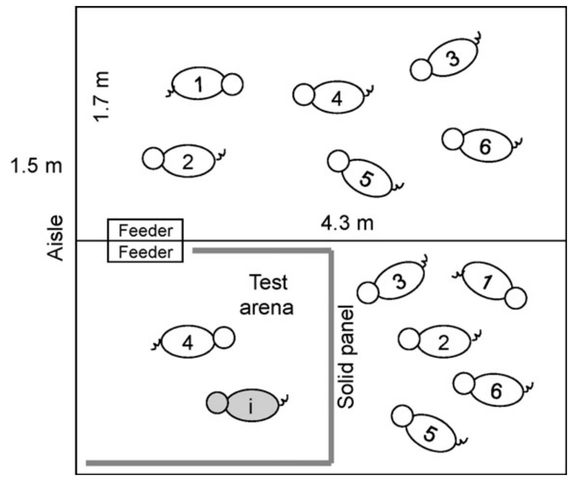

Fig. 1. Diagram of the test arena set in the home pens used for the resident–intruder test. A mobile solid white panel (grey lines) covered both sides and the back half of the pen while each animal was individually tested. In each pen of six gilts (numbered 1 through 6), at both 3 and 6 months of age, the resident pig (e.g. pig number 4) was introduced to an unfamiliar intruder pig (pig letter i) for a maximum of 300 s or until an attack (bite) happened. The latency to the first attack was recorded and pigs were immediately separated to prevent escalation of fighting. While testing one gilt, the other pen mates in the pen were held behind the center panel until it was their turn to be tested.

The R–I tests were performed over two consecutive days (a test pair: Test day 1 and Test day 2) in order to evaluate for consistency of outcomes, at the last 2 days of the dietary feeding trials (days 6 and 7; from 08:00 to 10:30 h). All tests were continuously recorded using the ceiling-mounted cameras (Panasonic WV-CD110AE), a DVR system, in addition to a camcorder at ground level (DCR-TRV260, Sony, CA, USA); the videos were used for behavioural analysis of the tests. The intruder group comprised of 24 gilts that were unfamiliar and unrelated to the resident gilts. Body weights of resident and intruder gilts were obtained on the day prior to testing. Intruder gilts' body weights and age were balanced for the body weights and age of the respective resident gilts against which they were being tested. Overall, resident gilts were 3.9 ± 1.6 kg (mean ± SEM) heavier than the intruder gilts, but this difference was not statistically significant ($P > 0.10$). Each intruder gilt was exposed to four R–I tests (or two test pairs) at 3 months of age and another four R–I tests at 6 months of age. However, the intruders were tested against resident gilts from different pens during each test, and alternated between residents on the different dietary treatments (CTL and high-TRP fed). At the time of testing, the designated intruder gilt was moved from its home pen by an experienced handler into the test arena, which took approximately 3 min.

The beginning of each test was established as the moment the intruder gilt entered the home pen of the resident gilt. A maximum of 300 s was allowed as the testing period. An attack was defined as a physical bite or a sequence of bites initiated by either the resident or the intruder gilt. Latency (s) to the first attack and the outcome of each test were recorded. Attacks initiated by the resident gilts were called 'resident attack' (RA), and the attacks initiated by the intruder gilts were counted as 'intruder attack' (IA). If no attack happened within the 300-s period, the test was terminated by removal of the intruder gilt from the test arena, and the outcome was classified as 'non-attack' (NA). Pigs were not allowed to fully engage in a two-sided fight as the test was also terminated and animals separated once one of the gilts, either resident or intruder, had initiated an attack. For the statistical analysis of latency to the first attack displayed during the R–I tests, only RA results were included since the study focused on the aggressiveness of the resident and not the intruder gilt. However, in order to take into account attacks initiated by intruder gilts, as well as non-attacks, the frequencies of RA, IA and NA (test outcomes) were also analyzed.

2.8. Statistical analysis

Data from gilts within a pen ($N = 8$) were averaged for statistical analyses of growth performance, home pen aggression, and latency to attack. One gilt assigned to the social handling treatment and belonging to block 1 died following weaning, and thus, data from 47 gilts were used in the analyses of blood TRP and 5-HT. Experimental blocks 1 and 2 were part of a complete balanced block design with social handling (non-handled and handled) and dietary treatment (CTL and high-TRP feeding) as factors. All the variances were estimated, model assumptions (e.g. normality) assessed (PROC UNIVARIATE, SAS Institute, Inc., Cary, NC, USA), and data transformed when appropriate. Log transformation was applied to blood concentrations of TRP and 5-HT (except 5-HT data for 6-month-old gilts), and square root and arcsine transformations (square root (behaviour or posture percentage /100) × 57.275779) were used for the time-budget behaviour and posture data (except stand data for both 3- and 6-month-old gilts). A 2 × 2 factorial arrangement of dietary treatment and social handling within age (3 and 6 months) was computed using multivariate mixed models using SAS software (PROC MIXED, SAS Institute, Inc.). For the analyses of ADG, body weights, home pen aggression, and latency to attack, the statistical model included terms for the fixed effects of dietary treatment, social handling, day, and their interactions, and block; the random effect included the interaction of dietary treatment and handling nested within pen (pen(dietary treatment × social handling)). Analyses of blood TRP and 5-HT data were computed with a model including terms for the fixed effects of dietary treatment, social handling, day, and their interactions, pen(dietary treatment × social handling), its interaction with day, and block; the random effects included pig(pen × dietary treatment × social handling) and its interaction with day. Main fixed effects and all possible interactions were computed depending on the significance of the higher order interactions, and *P*-values of pair-wise comparisons were adjusted by Tukey's post hoc test. Repeated measurements of day were included for the analyses of blood concentrations of TRP and 5-HT, time-budget behaviour and the R–I test data. Mantel–Haenszel chi-square distribution test was used to analyze the R–I test categorical outcomes (RA, IA and NA) according to day of testing, dietary treatment and social handling, and row mean statistical scores are reported. Non-transformed means, but also transformed means when appropriate, their respective standard errors of the means (SEM) and *P*-values are presented in the text, or in tables and figures. Mean differences with $P\text{-value} < 0.10$ were considered tendencies towards significance whereas, the mean differences with $P\text{-value} < 0.05$ were considered statistically different.

3. Results

3.1. Blood TRP and 5-HT profile

Blood profile of 3-month-old gilts. In relation to baseline (day 1, pre-start feeding), gilts fed the high-TRP diet nearly tripled blood TRP concentration (dietary treatment × collection day, $F_{1,39} = 189.69$; $P < 0.0001$), with 5-HT concentration increasing by 20.3% when compared to the CTL-fed gilts (dietary treatment × collection day, $F_{1,39} = 14.07$; $P = 0.0006$; Table 4). There was no effect of social handling on blood TRP and 5-HT concentrations either pre- or post-high-TRP feeding ($F_{1,39} = 0.67$; $P = 0.418$).

Blood profile of 6-month-old gilts. When fed to older gilts, high-TRP diet also increased blood TRP by 85.2%, while CTL-fed gilts had similar concentrations

Table 4
Peripheral blood concentration of the essential amino acid tryptophan and the amine serotonin in gilts fed either a control or a high-tryptophan diet.

Analyte[a] (μg/mL)	Age	Control diet[b]			High-TRP diet[c]			Pooled SEM
		Baseline[c]	Post-TRP feeding[a]	Change[d]	Baseline[c]	Post-TRP feeding[a]	Change[d]	
Tryptophan	3 months	1.71 (5.66)a	1.82 (6.33)a	11.8%	1.79 (6.17)a	2.84 (17.32)b	180.7%	0.04
	6 months	2.25 (9.72)a	2.43 (11.95)a	22.9%	2.21 (9.27)a	2.86 (18.00)b	85.2%	0.05
Serotonin	3 months	0.51 (1.77)a	0.51 (1.78)a	0.56%	0.46 (1.72)a	0.67 (2.07)b	20.3%	0.08
	6 months	1.19a	1.10a	−7.6%	1.17a	1.32a	12.8%	0.10

Within a row and dietary treatment, means without a common letter differ ($P < 0.05$).
[a] Where log transformation was applied, the respective back transformed means are presented between parentheses.
[b] Both diets were fed to the experimental gilts *ad libitum* for a period of 7 days. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet.
[c] Baseline peripheral blood concentrations correspond to sampling carried out at the same day but immediately prior starting feeding the experimental diets to the maternal line gilts (day 1). Post-feeding concentrations correspond to the blood sampling performed at the last day of the feeding trial (day 7).
[d] Percent changes correspond to differences between untransformed means. A positive percent value indicates an increase in relation to baseline, while a negative percent value indicates a decrease in relation to baseline concentration (day 1).

(treatment × collection day, $F_{1,39} = 27.27$; $P < 0.0001$; Table 4). However, there were no significant changes in the peripheral concentration 5-HT regardless of the dietary treatment applied; both high-TRP and CTL-fed gilts had similar blood 5-HT concentrations ($F_{1,39} = 0.80$; $P = 0.375$; Table 4). Again, there was no effect of social handling on blood TRP ($F_{1,39} = 1.19$; $P = 0.282$) and 5-HT ($F_{1,39} = 0.00$; $P = 0.964$) concentrations either pre- or post-high-TRP feeding trial.

3.2. Growth performance

3.2.1. Growth performance of 3-month-old gilts

The ADG during the feeding trial of the grower gilts was significantly affected by the interaction of dietary treatment × social handling ($F_{1,3} = 13.32$; $P = 0.035$) as positive social handling was associated with an increase in ADG in CTL-fed gilts (Fig. 2). Body weights of gilts assigned to high-TRP and CTL feeding were similar prior to the start the dietary feeding at day 1 (42.7 kg and 39.7 ± 1.4 kg, respectively; $F_{1,3} = 4.56$; $P = 0.122$). But, high-TRP fed gilts showed a statistical trend for higher body weights compared to CTL-fed gilts at the end of the trial or at day 7 (48.1 kg vs. 44.9 ± 0.8 kg, respectively; $F_{1,3} = 6.68$; $P = 0.082$). Body weights of non-handled and handled gilts were not different at neither day 1 (40.7 kg and 41.8 ± 1.4 kg, respectively, $F_{1,3} = 0.49$; $P = 0.536$), nor at day 7 (45.8 kg and 47.2 ± 1.5 kg, respectively; $F_{1,3} = 1.11$; $P = 0.370$).

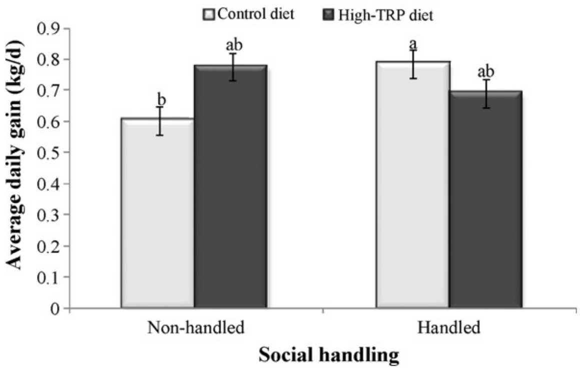

Fig. 2. Average daily gain of 3-month-old maternal line gilts subjected to a positive social handling protocol and fed either a control or a high-tryptophan (TRP) diet *ad libitum* for 7 days. The high-TRP diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet. Social handling was performed 3 days per week, starting when gilts were 45 days and lasted until they were 6 months of age. From 45 days to 3 1/2 months, handling of gilts lasted 6 min per pen per day. [a,b]$P < 0.05$.

3.2.2. Growth performance of 6-month-old gilts

The ADG was similar regardless of the dietary treatment (CTL = 0.83 kg/day vs. high-TRP = 0.74 ± 0.06 kg/day; $F_{1,3} = 0.34$; $P = 0.599$) or social handling (non-handled = 0.796 kg/day vs. handled = 0.78 ± 0.06 kg/day; $F_{1,3} = 0.00$; $P = 0.952$) applied to the finisher gilts. Gilts assigned to the CTL and high-TRP diets had similar body weights prior to start the dietary feeding (day 1, CTL = 108.2 kg vs. high-TRP = 106.6 ± 2.3 kg; $F_{1,3} = 0.20$; $P = 0.683$) and on the last day of the dietary feeding trial (day 7, CTL = 114.0 kg vs. high-TRP = 111.8 ± 2.4 kg; $F_{1,3} = 0.62$; $P = 0.490$). There was also no evidence of social handling effect on the pre-trial body weight (non-handled = 107.2 kg vs. handled = 107.6 ± 2.3 kg; $F_{1,3} = 0.00$; $P = 0.956$) or the post-trial body weight (non-handled = 112.7 kg vs. handled = 113.1 ± 2.4 kg; $F_{1,3} = 0.00$; $P = 0.978$).

3.3. Time-budget behaviour

3.3.1. Time-budget behaviour of 3-month-old gilts

Inactive behaviour. High-TRP-fed grower gilts spent significantly more time inactive than CTL-fed gilts (52.2 (60.1%) vs. 47.8 (53.4%) ± 0.8, respectively; $F_{1,3} = 15.10$; $P = 0.030$). Gilts that were not exposed to the social handling showed a statistical trend for greater inactivity compared to handled and non-handled CTL-fed gilts, but did not differ from handled TRP-fed gilts (dietary treatment × social handling; $F_{1,3} = 5.89$; $P = 0.093$; Table 5). Day of feeding had no effect on behavioural activity of the experimental gilts ($F_{4,415} = 0.20$; $P = 0.938$).

Active behaviours and postures. High-TRP diet feeding tended to reduce the time spent walking (2.1 (2.5%) vs. 2.4 (3.4%) ± 0.1, respectively; $F_{1,3} = 7.36$; $P = 0.073$), and nosing/rooting (16.8 (11.1%) vs. 18.9 (12.5%) ± 1.0, respectively; $F_{1,3} = 5.98$; $P = 0.092$) in grower gilts compared to the CTL-fed gilts. There was no evidence for differences in time spent alert, nose/rooting, sham-chewing, engaging in non-aggressive interactions, feeding, drinking or eliminating in relation

Table 5
Mean percentages of time spent in various behaviours and postures of 3-month-old gilts.

Behaviour (%)[a]	Control diet[b]		High-TRP diet[b]		Pooled SEM	Dietary treatment		Social handling	
	Handled[c]	Non-handled	Handled[c]	Non-handled		$F_{1,3}$ value	P-Value	$F_{1,3}$ value	P-Value
Inactive	48.5 (54.5)x	46.7 (52.4)x	50.5 (57.3)xy	53.4 (62.7)y	1.0	15.10	0.030	0.24	0.658
Alert	13.8 (7.6)	14.2 (7.4)	12.8 (7.2)	12.2 (6.4)	1.7	4.10	0.136	0.03	0.878
Walk	2.4 (4.0)	2.5 (2.9)	2.2 (2.9)	2.1 (2.2)	0.3	7.36	0.073	0.01	0.917
Nose/root	17.4 (10.9)	20.5 (14.1)	16.8 (11.4)	16.9 (10.8)	1.1	5.98	0.092	3.43	0.161
Sham-chew	0.7 (0.2)	0.9 (0.3)	1.1 (0.4)	0.2 (0.1)	0.6	0.06	0.826	0.83	0.430
Non-aggressive int.	11.6 (6.1)	9.5 (4.5)	10.0 (4.9)	9.4 (4.3)	0.9	1.42	0.319	3.44	0.161
Aggressive int.	0.6 (0.2)	0.6 (0.2)	0.4 (0.2)	0.9 (0.3)	0.3	0.01	0.914	0.50	0.529
Feed	20.4 (14.0)	22.5 (16.5)	20.3 (14.4)	19.6 (12.5)	1.3	0.90	0.414	0.19	0.689
Drink	3.9 (1.4)	3.4 (1.2)	3.4 (1.0)	1.9 (0.8)	1.1	0.87	0.420	1.03	0.384
Eliminate	0.9 (0.3)	0.4 (0.1)	0.5 (0.2)	0.6 (0.2)	0.4	0.03	0.879	0.19	0.693
Posture (%)[a]									
Stand	36.9a	39.4a	34.5ab	29.8b	3.6	11.00	0.045	0.42	0.561
Sit	5.4 (2.0)	4.6 (1.8)	7.5 (3.3)	8.4 (3.6)	1.4	2.23	0.232	0.00	0.991
Lie	51.6 (59.6)ab	48.0 (55.1)a	53.7 (61.9)b	55.7 (66.3)b	1.9	11.43	0.043	0.32	0.609

[a,b]Within a row, means without a common letter differ ($P < 0.05$; dietary treatment × social handling F and P-values are presented in the text). [x,y]Within a row, means without a common letter differ ($0.10 < P > 0.05$; dietary treatment × social handling F and P-values are presented in the text).

[a] Means shown as percentage of time spent performing the behaviour/posture for a 12-h interval (06:00–18:00 h). Where square root transformation (walk) or arcsine transformation (all other behaviours and postures, except stand) was applied, the respective back transformed means are presented between parentheses.

[b] Both diets were fed to the experimental gilts *ad libitum* for a period of 7 days. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet.

[c] Social handling was performed 3 days per week, starting when gilts were 45 days and lasted until they were 6 months old. From 45 days to 3 1/2 months, the handling of gilts lasted 6 min per pen per day; from 3 1/2 until 6 months of age, handing of gilts lasted 3 min per pen per day.

to high-TRP feeding or long-term positive social handling of 3-month-old gilts ($P > 0.10$; Table 5). In relation to postures, the high-TRP fed gilts spent significantly less time standing (32.1% vs. 38.1 ± 1.3%, respectively; $F_{1,3} = 11.00$; $P = 0.045$), and in turn they spent more time lying down compared to CTL-fed gilts (54.7 (64.1%) vs. 49.8 (57.3%) ± 1.1, respectively; $F_{1,3} = 11.43$; $P = 0.043$). High-TRP feeding did not have an effect on time spent sitting, nor there were differences in postures were observed in consequence of the social handling procedure ($P > 0.10$; Table 5). Day of feeding had no effect on any behavioural activity or postures of the experimental gilts ($F_{4,415}$ = all values > 1.95; $P > 0.10$).

3.3.2. Time-budget behaviour of gilts at 6 months of age

Inactive behaviour. There were no significant differences in inactivity associated with high-TRP feeding or social handling of gilts at 6 months of age ($P > 0.10$; Table 6). Day of feeding had no effect on behavioural activity of the experimental gilts ($F_{4,415}$ = all values > 1.95; $P > 0.10$).

Table 6
Mean percentages of time spent in various behaviours and postures of 6-month-old gilts.

behaviour (%)[a]	Control diet[b]		High-TRP diet[b]		Pooled SEM	Dietary treatment		Social handling	
	Handled[c]	Non-handled	Handled[c]	Non-handled		$F_{1,3}$ value	P-Value	$F_{1,3}$ value	P-Value
Inactive	53.0 (63.3)	52.3 (62.1)	54.7 (65.0)	55.4 (65.4)	4.4	0.32	0.613	0.00	0.990
Alert	7.5 (3.4)	6.8 (2.8)	6.8 (3.0)	6.6 (2.8)	1.2	0.22	0.671	0.21	0.680
Walk	3.3 (1.2)	4.1 (1.5)	3.8 (1.4)	3.9 (1.4)	0.6	0.06	0.815	0.47	0.543
Nose/root	13.5 (8.6)	13.5 (8.7)	11.9 (7.1)	15.2 (10.3)	1.3	0.00	0.979	2.63	0.203
Sham-chew	5.6 (2.3)	6.4 (2.6)	3.7 (1.3)	6.0 (2.2)	0.9	1.35	0.329	2.50	0.212
Non-aggressive int.	4.1 (1.4)	4.8 (1.9)	5.6 (2.5)	5.7 (2.5)	1.6	0.71	0.461	0.09	0.788
Aggressive int.	0.4 (0.1)	0.3 (0.1)	0.5 (0.2)	0.3 (0.1)	0.2	0.05	0.833	0.28	0.632
Feed	15.0 (9.2)	15.5 (9.6)	15.0 (9.3)	14.2 (8.3)	1.1	0.26	0.642	0.03	0.867
Drink	4.4 (1.6)	4.0 (1.4)	2.9 (0.9)	3.0 (1.0)	0.6	3.72	0.149	0.07	0.810
Eliminate	0.1 (0.0)	1.2 (0.4)	0.4 (0.1)	0.8 (0.2)	0.5	0.02	0.892	9.59	0.053
Posture (%)[a]									
Stand	28.7	31.4	29.9	26.8	1.2	0.03	0.880	1.89	0.263
Sit	9.0 (4.3)a	6.3 (2.8)ab	5.1 (1.9)b	7.4 (3.3)ab	2.7	3.36	0.164	0.06	0.830
Lie	55.5 (67.6)	54.8 (67.1)	56.9 (68.8)	58.4 (69.8)	4.9	0.27	0.638	0.01	0.923

[a,b]Within a row, means without a common letter differ ($P < 0.05$; dietary treatment × social handling F and P-values are presented in the text). [x,y]Within a row, means without a common letter differ ($0.10 < P > 0.05$; dietary treatment × social handling F and P-values are presented in the text).

[a] Means shown as percentage of time spent performing the behaviour/posture for a 12-h interval (06:00–18:00 h). Where arcsine transformation (all behaviours and postures, except stand) was applied, the respective back transformed means are presented between parentheses.

[b] Both diets were fed to the experimental gilts *ad libitum* for a period of 7 days. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet.

[c] Social handling was performed 3 days per week, starting when gilts were 45 days and lasted until they were 6 months old. From 45 days to 3 1/2 months, the handling of gilts lasted 6 min per pen per day; from 3 1/2 until 6 months of age, handing of gilts lasted 3 min per pen per day.

Table 7
Home pen aggression profile for replacement gilts at 3 and 6 months of age according to dietary treatment and positive social handling shown as the total number of agonistic interactions observed per day of feeding and the average number of bites and head-knocks per interaction.

	Dietary treatment[a]		Social handling[b]		Pooled SEM
	Control	High-TRP	Non-handled	Handled	
3 months					
Number of interactions	22.8a	14.8b	20.6	17.0	3.0
Bites per interaction	4.7	3.6	4.5	3.9	0.7
Head-knocks per interaction	2.5	2.5	2.8x	2.2y	0.2
Sum per interaction	7.2	6.2	7.3	6.1	0.6
6 months					
Number of interactions	10.2	7.2	9.6	7.7	2.0
Bites per interaction	2.6	2.9	2.7	2.8	0.5
Head-knocks per interaction	1.7	1.7	1.6	1.8	0.3
Sum per interaction	4.3	4.7	4.3	4.7	0.8

[a,b]Within a row and dietary treatments, means without a common letter differ ($P < 0.05$). [x,y]Within a row and social handling, means without a common letter differ ($P = 0.09$).

[a] Both diets were fed to the experimental gilts *ad libitum* for a period of 7 days. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet.

[b] Social handling was performed 3 days per week, starting when gilts were 45 days and lasted until they were 6 months old. From 45 days to 3 1/2 months, the handling of gilts lasted 6 min per pen per day; from 3 1/2 until 6 months of age, handing of gilts lasted 3 min per pen per day.

Active behaviours. There was a statistical tendency for non-handled gilts to spent more time eliminating (defecating and urinating) than socially handled gilts (1.0 (0.3%) vs. 0.2 (0.1%) ± 0.5, respectively; $F_{1,3} = 9.59$; $P = 0.053$). In relation to the other active behaviours recorded for the 6-month-old gilts, there were no significant differences associated with high-TRP feeding or social handling ($P > 0.10$; Table 6). In relation to postures, the dietary treatment × social handling interaction was significant for time spent sitting ($F_{1,3} = 10.79$; $P = 0.046$); handled gilts that were fed the high-TRP diet spent the least while handled CTL-fed gilts spent the greatest percentage of their time sitting ($P < 0.05$; Table 6). Non-handled gilts fed the high-TRP and CTL diets spent an intermediate percentage of their time sitting compared to those of handled gilts ($P > 0.10$; Table 6). There were no differences in time spent standing or lying in relation to high-TRP feeding or social handling for the 6-month-old gilts ($P > 0.10$; Table 6). Time spent in any specific posture was not affected by the day of TRP or CTL diet feeding ($F_{4,415}$ = all values > 1.95; $P > 0.10$).

3.4. Aggression in the home pen

3.4.1. Home pen aggression of 3-month-old gilts

The total number of agonistic interactions taking place in each pen per trial day was significantly lower in high-TRP compared to CTL-fed gilts ($F_{1,3} = 13.56$; $P = 0.035$; Table 7). The average number of bites per agonistic interaction was not affected by either the high-TRP feeding ($F_{1,3} = 1.04$; $P = 0.383$) or social handling ($F_{1,3} = 0.33$; $P = 0.603$; Table 7). Similarly, the average head-knock count per agonistic interaction was not affected by the dietary treatment ($F_{1,3} = 0.08$; $P = 0.800$); however, handled gilts showed a trend to display fewer head-knocks per agonistic interaction compared to non-handled gilts ($F_{1,3} = 5.93$; $P = 0.093$; Table 7).

3.4.2. Home pen aggression of 6-month-old gilts

The interaction of dietary treatment × social handling × day of feeding was significant for the number of bites per agonistic interaction ($F_{4,14} = 3.83$; $P = 0.026$); bites displayed by handled CTL-fed gilts tended to be higher on day 1 of the experiment (the highest counts) compared to day 3 (the lowest counts) of the feeding trial (day 1 = 5.30 vs. day 3 = 1.05 ± 1.06; $P = 0.06$); no other pair-wise comparison for the 3-way interaction was significant ($P > 0.10$). There was no main effect of dietary treatment ($F_{1,3} = 0.00$; $P = 0.996$) or social handling ($F_{1,3} = 0.19$; $P = 0.692$) on the number of head-knocks displayed per agonistic interaction (Table 7).

3.5. Resident–intruder (R–I) test

3.5.1. Aggressiveness of 3-month-old gilts

Frequency of attacks. On Test day 1, the high-TRP fed gilts displayed significantly fewer RA, but more IA and NA than CTL-fed gilts by the end of 300 s of testing ($\chi_{1,47} = 9.894$; $P = 0.002$; Fig. 5). However, there were no differences associated with dietary treatments in the frequency of attacks on Test day 2 ($\chi_{1,47} = 1.736$; $P = 0.188$;

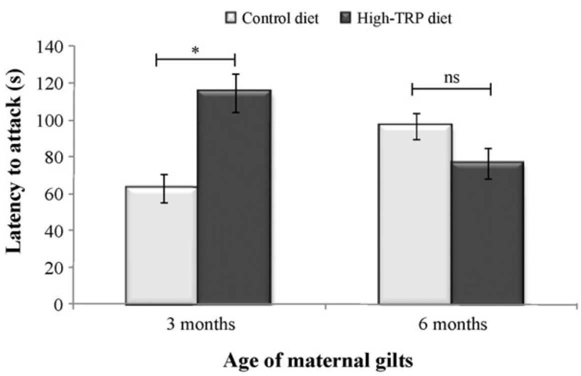

Fig. 3. Latency to the first attack initiated during a resident–intruder test carried out with 3 and 6 months old maternal line gilts that were fed either a control or a high-tryptophan (TRP) diet *ad libitum* for 7 days. The high-TRP diet was provided at a 250% inclusion rate of the TRP concentration available in the control diet. Shorter latency to attack is indicative of higher aggressiveness. *$P < 0.05$, ns = non-significant.

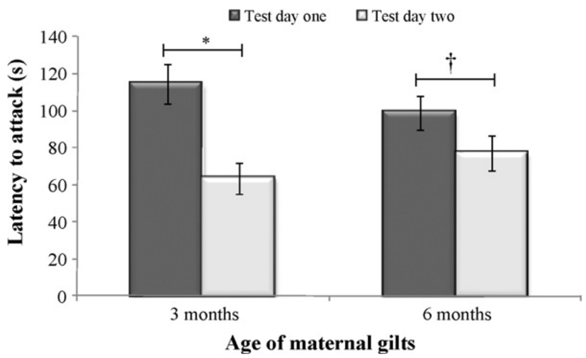

Fig. 4. Latency to the first attack (e.g. bite) displayed by 3 and 6 months old maternal line gilts according to the day of the resident–intruder test. Shorter latency to attack is indicative of higher aggressiveness. $^{*}P < 0.05$, $^{\dagger}P = 0.09$.

Fig. 5). Social handling of gilts did not affect the frequency of attacks and non-attacks carried out during testing ($\chi_{1,94} = 0.949$; $P = 0.330$). Overall, RA initiated by non-handled gilts totaled 77.1%, while intruders encountering non-handled gilts (IA) made up 12.5% of the total attacks displayed; NA totaled 10.4% of the encounters' outcomes. Resident gilts subjected to social handling initiated 65.2% of RA, were attacked by the intruder pig 15.2% (IA), and 19.6% of the encounters' outcomes were NA.

Latency to the first attack. Gilts fed the high-TRP diet took significantly longer to initiate an attack against the intruder gilt compared to CTL-fed gilts ($F_{1,3} = 16.56$; $P = 0.027$; Fig. 3). Overall, gilts showed a shorter latency to attack on the second day (Test day 2) compared to the first day (Test day 1) of R–I testing ($F_{1,57} = 12.20$; $P = 0.0009$; Fig. 4). No differences in latency to attack between non-handled and handled gilts at 3 months of age were observed (83.4 ± 8.4 s vs. 94.6 ± 9.7 s, respectively; $F_{1,3} = 0.75$; $P = 0.450$).

3.5.2. Aggressiveness of 6-month-old gilts

Frequency of attack. The frequency of attacks and non-attacks displayed by finisher gilts was not affected by the dietary treatment ($\chi_{1,94} = 2.131$; $P = 0.144$), social handling ($\chi_{1,94} = 0.363$; $P = 0.547$) and/or by the day of the R–I test ($\chi_{1,94} = 0.020$; $P = 0.886$; Fig. 5).

Latency to the first attack. Only a trend towards significance was observed between the two consecutive days of R–I test, in which latency to attack at Test day 2 was slightly shorter compared to Test day 1 ($F_{1,58} = 2.97$; $P = 0.900$; Fig. 4). There was no evidence of latency to attack being affected by the dietary treatment ($F_{1,3} = 5.53$; $P = 0.100$; Fig. 3) or social handling (non-handled = 85.1 ± 6.9 s vs. handled = 91.0 ± 6.4 s; $F_{1,3} = 0.38$; $P = 0.583$) applied to the 6-month-old gilts.

4. Discussion

The current study demonstrated that a high-TRP diet fed to 3-month-old gilts for 7 days reduced both behavioural activity and agonistic interactions observed in the home pen, and aggressiveness when tested using the R–I test. However, this effect was not observed when the enhanced TRP diet was fed to gilts at 6 months of age. For the current study, the only difference between the control and the 250% TRP-enhanced diets, for both the grower and finisher phases, was the amount of TRP that substituted the starch portion in the control diet. Social handling, started when gilts were 45 days old and was carried out three times a week, had a positive effect on ADG in grower gilts, but not in finisher gilts, and when associated with the high-TRP feeding reduced sitting behaviour.

Tryptophan, acquired through dietary supplementation, crosses the blood–brain-barrier via transporter proteins and acts as the precursor for 5-HT synthesis in the serotonergic neurons (e.g. Fernstrom, 1974), and thus TRP intake positively correlates with 5-HT availability in pig brain (Meunier-Salaün et al., 1991; Henry et al., 1992; Adeola and Ball, 1992). Serotonin plays a major role in appetite, behavioural activity, and aggression regulatory mechanism, and its deficiency leads to intensification of aggressive behaviours in several animal species and humans (Miczek et al., 2002; Miczek and Fish, 2005;

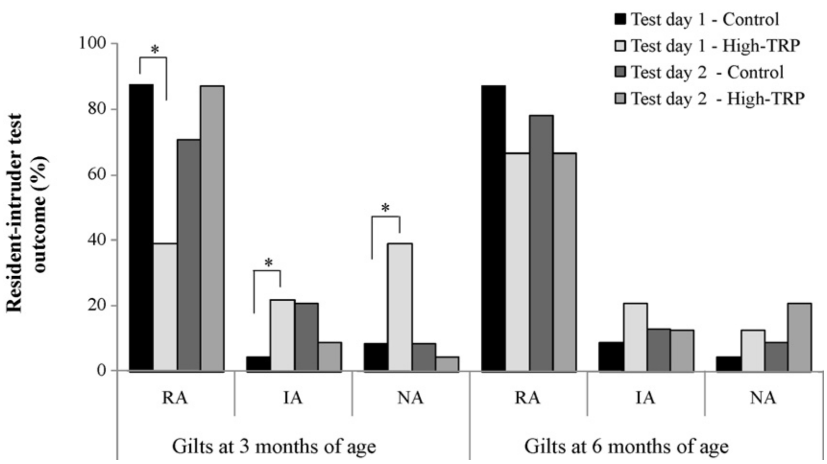

Fig. 5. Percentage of attacks carried out by the resident (RA) and intruder (IA) maternal line gilts at 3 and 6 months of age, and non-attacks (NA) displayed at day 1 and day 2 of the resident–intruder test. The high-tryptophan (TRP) diet was provided at a 250% inclusion rate of the TRP concentration available in the control (CTL) diet, both diets were fed *ad libitum* for 7 days. $^{*}P < 0.05$.

Linder et al., 2007). Hence, the main purpose of feeding the high-TRP diet to the experimental gilts was to indirectly raise brain availability of 5-HT. Finisher pigs fed TRP-supplemented diets at 2.5, 5 and 10 g/kg for 3, 5, 7 or 10 days had increased both the blood TRP levels and hypothalamus 5-HT concentration, which peaked at 5 days from the start of feeding (Adeola and Ball, 1992).

The selection of a 7-day feeding trial was intended to assure enough time for the high-TRP to exert any biological effects on the pigs' physiological and behavioural responses. A similar length of high-TRP feeding (7 days) was also adopted by Li et al. (2006) and Koopmans et al. (2005). The study by Adeola and Ball (1992), in addition to a pilot study carried out by our group, determined the concentration and length of TRP feeding for the current study. The pilot study consisted of feeding 3 and 6-month-old gilts, different from the ones used in the current study, with either a control diet, or diets with 200% and 400% TRP of the control diet for 7 days and determining daily blood TRP concentrations. During this pilot study, blood TRP concentration increased considerably within the first 24 h of feeding the TRP-enhanced diet. Nevertheless, in the present study, the lack of effect of day of feeding further suggests the idea that biological changes, which reflect in actual behavioural performance, may happen over several days of sustained TRP feeding.

The elevation in the blood TRP concentration observed in the experimental replacement gilts fed the high-TRP diet at both 3 and 6 months of age confirms the direct relationship between dietary and blood TRP concentrations previously reported in pigs (Meunier-Salaün et al., 1991; Adeola and Ball, 1992; Henry et al., 1992; Koopmans et al., 2006). However, when feeding the 250% TRP diet to gilts at 3 months of age, blood TRP concentration increased to almost 3-fold (180.7%) the baseline amount, while when feeding the same diet when gilts were 6 months of age, led the blood TRP concentration to increase at a lower amount (85.2%) relative to baseline. This reduction in change in the blood TRP concentration of the gilts at 6 months compared to 3 months of age may account for the lack of differences in behaviour observed between the dietary treatments. This result suggests the need for a TRP dietary inclusion rate greater than 250% for behaviour changes to occur in older gilts. There is also the potential for interference of sexual hormones in gilts reaching puberty on TRP requirement (Henry et al., 1992) and metabolism (Braidman and Rose, 1971), which may explain some of the lack of effects seen at the age of 6 months. Interestingly, the increases in blood TRP concentration only reflected in significant elevation of blood 5-HT concentration in 3-month-old gilts. It is worth noting that 5-HT brain concentrations were not measured in the current study because the pigs used were replacement maternal line gilts raised to stay in the herd. But, blood 5-HT concentration serves therefore as a proxy for neural 5-HT changes, instead of a direct measurement of brain concentrations.

Positive social interactions between humans and animals are beneficial to reduce stress that is associated with production practices, and can improve growth performance, productivity, and reproductive performance (Hemsworth et al., 1981; Hemsworth, 2007). In the current study, we also found that the long-term positive social handling (gentle touching, patting and low voice tone), administered to the gilts three times weekly for 6 min per pen, increased ADG of control-fed gilts when measured at 3 months. Accustoming gilts to human pleasant handling likely played a role in minimising their reaction to the environment and routine management practices during development hence enhancing growth performance. Whether non-handled experimental gilts were under any type of chronic stress that could have been reduced by the applied handling approach is questionable and should be verified, for instance, through measurements of stress hormones. Positive social handling imposed three times per week for 2 min in duration from 11 to 22 weeks of age, reduced chronic stress and improved growth rate of gilts that were interacting more with the handler (Hemsworth et al., 1981). In the absence of social handling, the high-TRP feeding may have played an immediate compensatory effect as it tended to increase weight gain of 3-month-old gilts (day 1 vs. day 7 body weights). The likely augmentation in brain serotonergic activity may have played a role in stimulating feed intake and consequently weight gain (Henry et al., 1996; Linder et al., 2007); though this positive effect of TRP feeding on body weight was not noted in nursery piglets fed a TRP-enhanced diet (Koopmans et al., 2006).

Tryptophan feeding above amounts required for optimal growth reduced overall behavioural activity, with some potential to decrease walking and pen exploration (nosing/rooting behaviour) in growing gilts, was an outcome anticipated by the positive relationship between TRP intake and 5-HT enhanced activity (Fernstrom, 1974; Linder et al., 2007). Long-term social handling instead had a reductive effect on time spent sitting in gilts at 6 months of age, but only when associated with feeding the high-TRP diet. Grower/finisher pigs receiving two and four times the required amount of TRP for 7 days spent more time lying down and less time feeding than the pigs receiving the normal amount of TRP (Li et al., 2006), and nursery piglets supplemented with dietary TRP for 10 days also spent more time lying and less time standing (Koopmans et al., 2006). A decrease in behavioural activity attenuates the odds for social interactions within the home pen and in turn may minimize the occurrence of agonistic interactions.

High levels of aggression can be deleterious to the animal's health and welfare because of injuries that may lead to death, and social stress (D'Eath and Turner, 2009). Tryptophan supplementation and thus activation of brain serotonergic activity is widely implicated in hindering hostility and aggression (Dougherty et al., 1999; Miczek et al., 2002; Miczek and Fish, 2005). The gilts used for this study remained with the same pen mates from weaning, forming a stable social group, and thus agonistic interactions (e.g. fights) were not very evident during behavioural observations. Nevertheless, feeding of the high-TRP diet markedly reduced the occurrence of agonistic interactions in the home pen of gilts at 3 months of age, hence becoming a valuable tool to help minimize aggression in group-housed pigs. This may be especially useful at specific times such as preceding mixing. A study by

Li et al. (2006) found no effect of enhanced TRP diet fed for 7 days to grower/finisher pigs on the incidence or frequency of aggressive interactions, but instead observed that the time spent fighting was significantly reduced in the TRP group. More significant differences in amount of aggressive interactions, and the actions displayed during each interaction in the home pen, may have been observed if the study had examined the effects of the high-TRP and social handling among groups of unfamiliar gilts—at mixing for instance (Stookey and Gonyou, 1994); nevertheless, this scenario could be partially simulated by using the R–I test.

In a group context, pigs engage in aggressive interactions with unfamiliar conspecifics to determine their social dominance and thereafter to compete for resources such as access to the feeder (Mendl et al., 1992; Erhard and Mendl, 1997). To mimic this paradigm of mixing unfamiliar animals, a common event in swine production, the R–I test, primarily used to predict aggressiveness in rodents (Kemble, 1993), has been adapted for pigs (Erhard and Mendl, 1997; D'Eath, 2002; D'Eath and Pickup, 2002) and was used in the current study. The latency for the resident pig to initiate an attack negatively correlates with its aggressiveness (Erhard and Mendl, 1997; D'Eath and Pickup, 2002; D'Eath, 2002). High-TRP feeding reduced significantly the aggressiveness of grower gilts as they took longer to attack the intruder pig, and on day 1 of the testing, initiated less attacks while receiving more intruder attacks. In contrast, grower barrows fed a high TRP:LNAA ratio diet for 7 days showed normal aggressive behaviours during social confrontations (Koopmans et al., 2005). A study by D'Eath et al. (2005) found fewer cells expressing 5-HT_{1A} mRNA, a serotonergic receptor involved in aggression regulation, in gilts that showed greater aggressiveness during the R–I test. The shorter latency to attack observed on the second day of testing compared to the first day, observed gilts at both 3 and 6 months of age, may be explained by the fact that the pigs can rapidly learn about the testing procedure and anticipate the outcome of the encounter when the opponent is an unfamiliar pig (D'Eath, 2002; D'Eath and Pickup, 2002).

In conclusion, diets with high-TRP concentration (high TRP:LNAA ratio) reduced behavioural activity and aggressiveness of grower gilts, and the serotonergic system may be mediating these effects. Meanwhile, pleasant long-term social handling increased ADG and reduced sitting behaviour without affecting other postures. It is intriguing that most effects related to high-TRP feeding were seen when gilts were 3 months of age and not observed when the same gilts were 6 months old. There may be a greater TRP requirement in finisher gilts in order for a behavioural effect to occur and this is an area that deserves further research. Nevertheless, short-term supplementation of high-TRP concentration in the diet could be used to reduce aggression at mixing, at least in grower pigs.

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